SCREENING FOR PATHOGENIC ESCHERICHIA COLI IN THE CHATTAHOOCHEE RIVER, COLUMBUS, GEORGIA

Micah A. Staples

COLUMBUS STATE UNIVERSITY

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MICAH A. STAPLES

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Thesis Advisor _____ Date 05/10/2016
Dr. Lauren King

Committee Member John R Davis Date 5/10/2016
Dr. John Davis

Honors College Dean Cindy Ticknor Date 5/10/16
Dr. Cindy Ticknor

Abstract. *Escherichia coli* testing is frequently used to indicate the possible presence of harmful pathogens, but *E. coli* itself can be pathogenic. Serotype O157:H7, enterohemorrhagic *E. coli*, is likely the most medically important pathogenic strain of *E. coli* in the United States. In this study, we surveyed for O157:H7 and three strains of another pathogenic subtype: enterotoxigenic *E. coli*. We isolated *E. coli* colonies from samples taken from the Chattahoochee River in Columbus, Georgia at two different locations, above and below wastewater outputs. We used PCR to test for the individual subtypes and then used a nested PCR protocol for the human, bovine, and avian ETEC. Our results show that most isolated *E. coli* strains are not O157:H7 or ETEC. However, nested PCR specific for human enterotoxigenic *E. coli* amplified target sequences in some tested colonies, which may indicate humans as a source of *E. coli* at our sample sites.

Acknowledgements

I would like to thank my research mentor Dr. Lauren King for all her help and inspiration. She kept me on track with her patience and enthusiasm, and I had a wonderful time working with her.

I would also like to thank Dr. John Davis for being my second reader, Dr. John Barone for statistics help, Scott Silvis for PCR advice, and everyone in the Columbus State University Biology Department for all the support.

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Introduction

Escherichia coli is a commensal bacterium that resides almost exclusively within the digestive tract of vertebrates. It has been used as a fecal pollution indicator for more than 100 years (Escherich, 1885). The vast majority of *E. coli* strains are not pathogenic, but the presence of *E. coli* is used to predict the presence of harmful organisms that are harder to detect. There are also pathogenic strains of *E. coli* associated with outbreaks of various diseases (Jiang et al. 2007). O157:H7 is the primary serotype associated with enterohemorrhagic *E. coli* (EHEC), which causes hemolytic uremic syndrome (HUS) (Cohen et al. 1992). HUS symptoms include anemia, bloody stool, vomiting, diarrhea, and renal failure. Although the source of infection is typically contaminated beef (Chapman et al. 1997), cases of infection due to swimming in waters with O157:H7 *E. coli* have been documented (Keene et al. 1994). Enterotoxigenic *E. coli* is another subset of pathogenic E. coli. There are many different ETEC strains associated with the digestive systems of various vertebrates. ETEC infection occurs most frequently in developing countries and is commonly referred to as traveler's diarrhea (Qadri et al. 2005). Enterotoxigenic *E. coli* are still present in the United States, but sanitary conditions typically prevent infection. Isolating ETEC and determining its source provides insight into the primary sources of fecal pollution for a given body of water. Host-specific toxin genes associated with ETEC have been identified and used in microbial source determination (Jiang et al. 2007). Toxin genes specific for stains found in humans, birds, dogs, cattle, and many other animals have been identified.

Isolating *E. coli* is beneficial when discerning the proportion of pathogenic strains to non-pathogenic strains. In this experiment, we used eosin-methylene blue (EMB) agar to

identify *E. coli* in water samples and subsequently isolate it. *E. coli* is easily differentiated on EMB agar because of the distinctive metallic-green sheen produced by colonies. Leininger et al (2001) indicated that EMB agar is effective at differentiating between *E. coli* and other coliforms (*Klebsiella, Enterobacter, Serratia,* etc.). In this study, we isolated *E. coli* using EMB, and subsequently used PCR to identify O157:H7 EHEC and host-specific ETEC stains (Fig. 1). Prior research indicates that birds are the primary source of ETEC (Jiang et al. 2007) and that beef cattle are the primary source of O157 strains (Chapman et al 1997). Therefore, we expect a higher proportion of the *E. coli* found above the output of treated sewage to be O157 strains and EHEC than those found below the output.

Materials and Methods

Sampling

Water samples were taken from two sites on the Chattahoochee River in Columbus, Georgia. One sample designated "above" was collected from a boat ramp behind the Columbus Georgia Convention and Trade Center. The "below" sample site is 3.0 miles downstream from the Columbus wastewater output, 7.94 miles downstream from the first sample site, and adjacent to a field that is part of Oxbow Meadows Environmental Learning Center (Figs. 1 and 2). Samples were taken at both sites on the same day once a week at between 10:00 AM and 12:00 PM. Water temperature and pH were taken at both sample locations using a Hach HQ30d portable multi-parameter meter. Flow rate data for the river was pulled from the US Geological Survey website, which is measured 0.5 miles downstream from the first sample site. Columbus Water Works provided rainfall data.

E. coli Isolation

We used membrane filtration to isolate *E. coli* from our water samples. Ten dilutions of 10 mL for each sample were suspended in 50 mL of phosphate buffered saline, then vacuumed onto a membrane filter and transferred to eosin methylene blue agar on 50mm plates. Plates were incubated for 2 hours at 37.5°C, then 22(+/-2) hours at 44.5°C. *E. coli* colonies were tallied for all plates, and reported in Colony Forming Units(CFU)/100 mL. Twenty *E. coli* isolates from each sample were chosen at random for PCR screening.

Nested PCR for ETEC Toxin Genes and PCR for O157:H7

For identification of enterotoxigenic *E. coli*, we used a nested PCR protocol for toxin genes specific for three sources: humans, birds, and cows (Jiang et al 2007). A first set of "outer" primers for each source were used in an initial round of PCR. Any samples that produced positive results on the outer round of PCR were used in the second "inner" PCR round. Products from our initial PCR reaction were used in another reaction with source-specific primers designed to be selective for an amplicon within the amplicon of our first reaction. For identification of serotype O157:H7 *E. coli*, we used a PCR primer specific for an O-antigen biosynthesis gene (Maurer et al. 1999). PCR products were visualized via gel electrophoresis

RESULTS

No isolates tested positive for O157:H7 or any of the ETEC strains. Some isolates produced positive results for the outer toxin gene region for human ETEC, but the inner human primer

was negative for all isolates tested. There was no significant difference in the amount of isolates positive for the human outer primer between our two sample sites (Fig. 3. 1-way ANOVA: $F_{1,13}$=0.083, p = 0.778). *E. coli* counts were significantly higher in samples taken below wastewater output than those taken above (Fig. 4. 1-way ANOVA: $F_{1,13}$=4.882, p = 0.047). Water pH was not significantly different between the two sample sites (Fig. 5. 1-way ANOVA $F_{1,13}$=0.251, p = 0.626). There was also no significant difference in water temperature between the two sample sites (Fig. 6. 1-way ANOVA $F_{1,13}$=0.067, p = 0.801). A regression model significantly predicted the value of *E. coli* concentration using river flow rate, and 29.4% of variation in *E. coli* concentration was caused by changing flow rate (Linear Regression Analysis F1,13=4.986, p = 0.045).

Discussion

Our results do not indicate the presence of any O157:H7 or enterotoxigenic *E. coli*. Cattle are the primary reservoir of O157:H7 *E. coli*, and the source of one of the ETEC strains we screened for. We screened a total of 280 *E. coli* colonies and found no trace of either pathogenic *E. coli* variant that have cattle reservoirs, so it is likely that cattle fecal contamination is low in this area. ETEC from the three sources surveyed and O157:H7 *E. coli* are likely well managed by current wastewater treatment procedures. However, our method only screens for enterotoxigenic from of *E. coli* in birds, cows, and humans. The inner human ETEC primer produced positive results for some tested colonies. This may indicate humans as a primary source of *E. coli*, but further investigation is required to determine if that is probable. The first step of the nested PCR protocol provides a variety of products, due to the lack of a highly

specific binding site for the desired region. The varied products can then be tested for the desired gene. While positive results for the first step in our human ETEC nested PCR protocol may indicate humans as the primary source of contamination, non-specific binding of the PCR primer may be to blame. While the first step of the nested PCR protocol was not positive for the outer primers for bird and cow ETEC, it does not rule out birds or cows as sources of *E. coli*. *E. coli* counts were significantly higher below wastewater output than above. There was no significant difference in water temperature or pH between our two sites, indicating similar conditions for survivability. Columbus wastewater, Wercoba Creek and Phenix City wastewater have outlets between our two sites that could contribute to the amount of *E. coli* in the Chattahoochee. Columbus treated wastewater output is much lower than that of the river, so one or both of the other sources are more likely to increase the concentration of *E. coli* in the river. River flow rate was positively associated with *E. coli* concentration, which is consistent with prior research (Ouattara et al.). We found no ETEC or EHEC at our level of sampling, so future research may focus on increased sample load, testing on a water volume basis for pathogenic *E. coli* instead of isolating individual colonies. It is also possible that there are pathogenic *E. coli* variants in the Chattahoochee that we did not test for. Future endeavors may focus on different strains and pathotypes.

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FIGURES

Figure 1. Sample sites in Columbus, Ga. A. Sample site above Phenix City and Columbus outflows. B. Sample site below both outflows. Orange arrows indicate site where grab sample were taken.

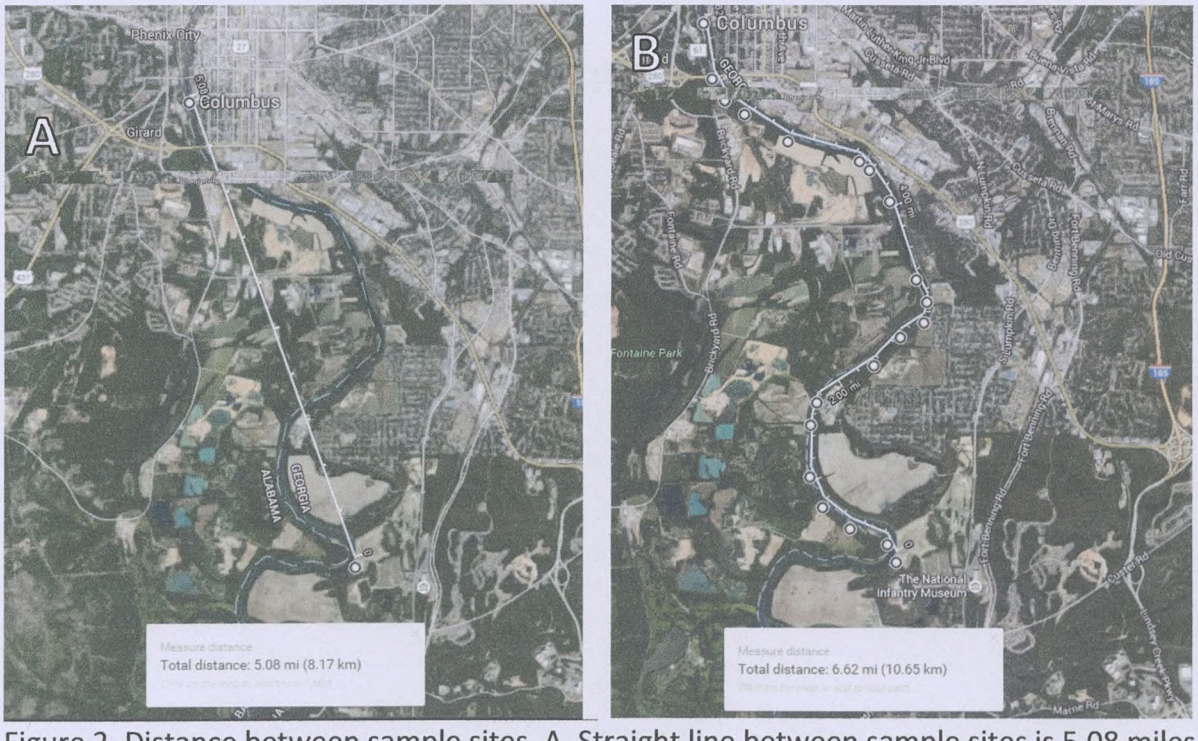

Figure 2. Distance between sample sites. A. Straight line between sample sites is 5.08 miles. B. 6.62 miles of river flow between sample sites.

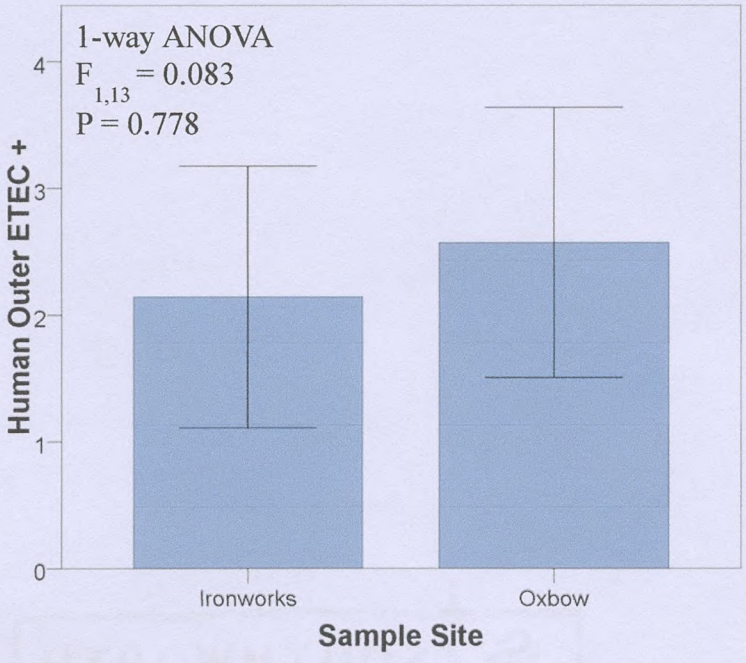

Figure 3. Mean (+/- 1 S.E.) *E. coli* colonies positive for human outer ETEC primer at two sample sites.

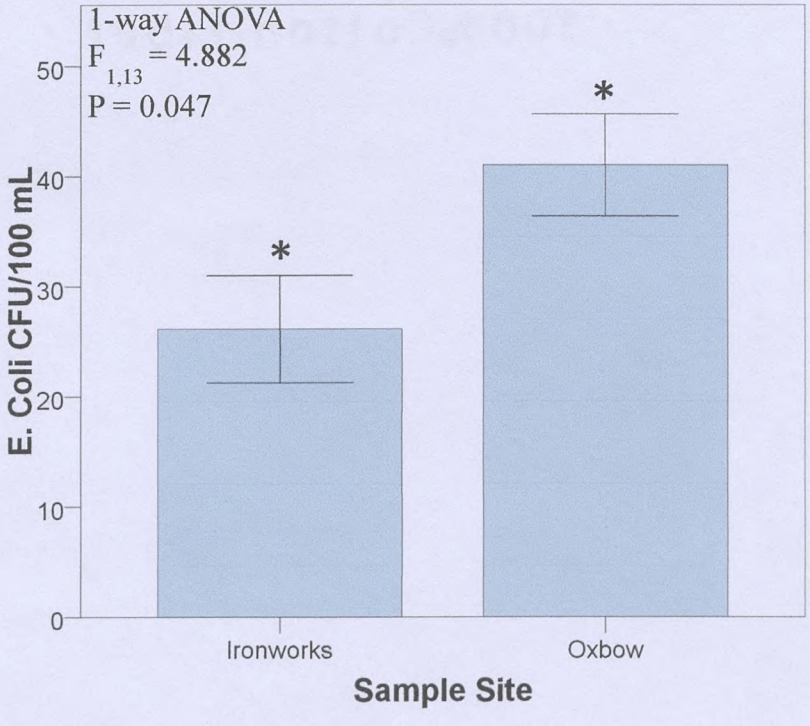

Figure 4. Mean (+/- 1 S.E.) *E. coli* concentration at two sample sites. Asterisk denotes statistical significance.

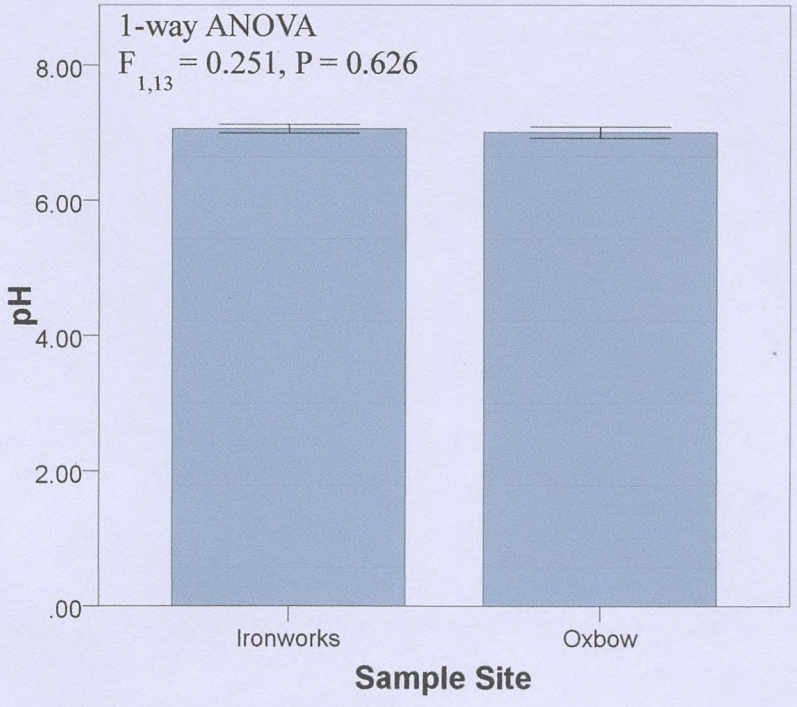

Figure 5. Mean (+/- 1 S.E.) water pH at two sample sites.

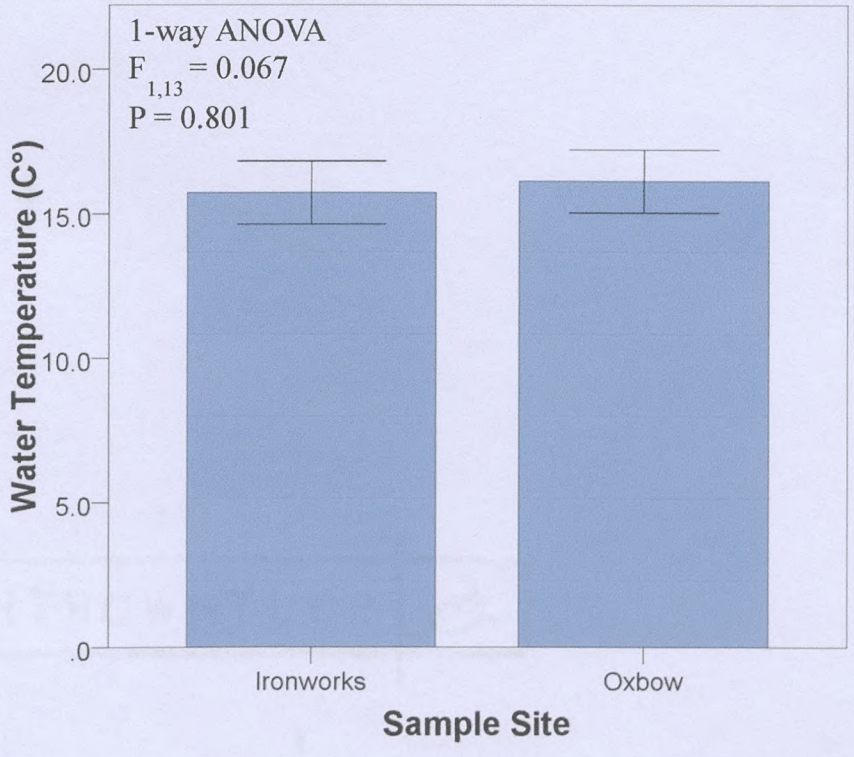

Figure 6. Mean (+/- 1 S.E.) water temperature at two sample sites.

TABLES

Table 1. List of PCR primers.

O157:H7	Forward 5'-CGTGATGATGTTGAGTTG-3' Reverse 5'-AGATTGGTTGGCATTACTG-3'	
Human ETEC	Outer Forward 5'-TGTATTGTCTTTTTCACC-3' Outer Reverse 5'-CATCATCAGAATCAGAAC-3'	Inner Forward 5'-CSCTCAGGATGCTAAACCAG-3' Inner Reverse 5'-TTAATAGCACCCGGTACAAGC-3'
Bird ETEC	Outer Forward 5'-CAACCTCTAACGGAAGTACC-3' Outer Reverse 5'-ATAAACGGGCCTCTATCACG-3'	Inner Forward 5'-CAGGCGGACAATAAAGGACAGG-3' Inner Reverse 5'-AGCACGGCACCATAATCTGC-3'
Cow ETEC	Outer Forward 5'-GGGTGTGCATTTCAGCGAC-3' Outer Reverse 5'-CGTCCACCCGGAATATACCA-3'	Inner Forward 5'-GCATGGAGAAAGAGATGAGC-3' Inner Reverse 5'-CTTACCACATAGATCCCACG-3'